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United States Patent Application *20040106186*
Kind Code **A1**
Horn, Jurgen **June 3, 2004**

Gamma-sterilisable nutrient medium based on casein soya peptone agar

Abstract

A gamma-sterilisable nutrient medium based on casein soya peptone agar for the detection of microorganisms in hydrogen peroxide-bearing air or on hydrogen peroxide-bearing surfaces, with a content of between 2 and 10% by weight of sodium thioglycolate, between 5 and 20% by weight of sodium thiosulfate and between 10 and 30% by weight of sodium disulfite in each case with respect to the agar. Preferably the agar used is microbial content test agar and the nutrient medium may contain between 0.1 and 0.25% by weight of sodium pyruvate with respect to the agar. If bromocresol purple and bromocresol violet are used as pH-indicators the nutrient medium may also contain polyvinylpyrrolidone.

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Claims

What is claimed is

1. A gamma-sterilisable nutrient medium- based on casein soya peptone agar for the detection of microorganisms in a hydrogen peroxide-bearing situation including the addition of between 2 and 10% by weight of sodium thioglycolate, between 5 and 20% by weight of sodium thiosulfate and between 10 and 30% by weight of sodium disulfite in each case with respect to the agar.

2. A gamma-sterilisable nutrient medium as set forth in claim 1 containing between 0.1 and 0.25% of sodium pyruvate with respect to the agar.

3. A gamma-sterilisable nutrient medium as set forth in claim 1 including at least one of bromocresol purple and bromocresol violet as a pH-indicator and between 10 and 50% by weight of polyvinylpyrrolidone with respect to the agar.

4. A gamma-sterilisable nutrient medium as set forth in claim 3 wherein the content of polyvinylpyrrolidone with respect to the agar is between 30 and 45% by weight.

5. A gamma-sterilisable nutrient medium as set forth in claim 1 including bromothymol blue as a pH-indicator and between 10 and 50% by weight of polyvinylpyrrolidone with respect to the agar.

6. A gamma-sterilisable nutrient medium as set forth in claim 5 wherein the content of polyvinylpyrrolidone with respect to the agar is between 30 and 45% by weight.

7. A gamma-sterilisable nutrient medium as set forth in claim 1 containing between 20 and 50% of morpholinopropane sulfonic acid and between 50 and 80% of phosphate buffer with respect to the total amount of buffer.

8. A gamma-sterilisable nutrient medium as set forth in claim 1 wherein microbial content test agar is used as the agar.

9. A gamma-sterilisable nutrient medium as set forth in claim 1 including at least one selected from the group consisting of betaine, glycine, cystine, proline and asparagine.

10. A gamma-sterilisable nutrient medium as set forth in claim 1 wherein the hydrogen peroxide-bearing situation is hydrogen-peroxide bearing air.

11. A gamma-sterilisable nutrient medium as set forth in claim 1 wherein the hydrogen peroxide-bearing situation is a hydrogen peroxide-bearing surface.

Description

FIELD OF THE INVENTION

[0001] The invention relates to a gamma-sterilisable nutrient medium based on casein soya peptone agar for the detection of microorganisms in hydrogen peroxide-bearing air or on a hydrogen peroxide-bearing surface.

[0002] More particularly this can be used for the detection of microorganisms such as bacteria, yeasts and fungi.

BACKGROUND OF THE INVENTION

[0003] Hydrogen peroxide can be used for fumigating isolators or entire rooms in order to destroy microorganisms which are possibly to be found therein. The hydrogen peroxide in gas form condenses on the fumigated surfaces, as a between 30% and 35% saturated solution. Prior to the start of manufacturing procedures, quality control investigations in respect of sterility or other operations in the fumigated clean room areas, they are sterily ventilated, whereafter levels of concentration of between 0.3 and 6 ppm, in general below 1 ppm, of germs remain in the air. Investigations in regard to such air-borne germs which are possibly still present is effected with air-borne germ collecting devices operating on the basis of the impaction or rotation principle, with the germ-bearing particles being deposited on agar surfaces.

[0004] Surprisingly the small amounts of hydrogen peroxide vapors are concentrated in the course of collecting 1000 liters of air in casein soya peptone agar, in accordance with United States Pharmakopoeae, 8th Supplement, USP-NF, <1116>, 4426-4431, on concentrations of over 100 ppm in agar. Spores are already restrained by levels of hydrogen peroxide concentration of 10 ppm and vegetative cells and microorganisms are already restrained by an even more markedly lower concentration of hydrogen peroxide. That adversely affects to a considerable degree the detectability of microorganisms which are still present.

[0005] Normal agar media have a contamination rate of about 0.1%, that is to say 1 non-sterile unit among 1000, and therefore do not comply with the notion of sterility which allows only one non-sterile unit among 10.sup.6 (one million). As the media used for the investigatory procedure are employed to investigate clean rooms which were previously rendered germ-free by fumigation, it would be desirable for no germs to be introduced with the agar materials for testing for freedom from germs, and therefore the expectation is for sterile media.

[0006] For conducting detection procedures of the above-indicated kind, it is possible to use media such as the Baird-Parker medium (Journ. Applied Bacteriology 25:12, 1962, Baird-Parker) with 1% of sodium pyruvate, being a sodium salt of pyruvic acid or 2-oxopropionic acid, which inter alia are suitable for the isolation of Staphylococcus aureus after heat damage or storage of frozen or dried vegetative cells. The disadvantage of Baird-Parker agar is the low level of stability and durability of the finished agar medium.

[0007] The addition of catalase to this and other media for improving the growth of bacteria from the air is also known, in which respect reference may be made to Journ. Applied and Environmental Microbiology 57: 2775-2776, 1991, Balkumar Marthi. Catalase breaks down hydrogen peroxide into water and oxygen. Catalase however is inactivated at 55.degree. C., which presupposes drawing off agar at markedly below 55.degree. C. That however is scarcely a viable option because of gelling of the agar at temperatures around 50.degree. C. In addition the oxygen resulting from the hydrogen peroxide causes bubbles and cracks in the agar, which causes extreme difficulty in detecting colonies which grow on the agar.

[0008] Also known is D/E-agar which neutralises a wide range of antiseptic and disinfecting chemicals including quaternary ammonium compounds, phenol, iodine and chlorine compounds, mercury (Merthiolate), formaldehyde and glutaraldehyde (Difco Handbook, D/E-Agar). That Difco Handbook does not describe neutralisation of hydrogen peroxide. The disadvantage of D/E-agar is the short durability life of the ready agar medium of only about two and a half months and the changes in the medium in the event of radiation doses of 16-25 kgray, which are necessary for reliable gamma-sterilisation.

[0009] In addition D/E-agar is not very stable in respect of pH and at a pH of 7.6.+-.0.2, is already of a very high pH-value. Upon drifting towards even higher pH-values such as 8.0, namely only 0.2 above the upper limit of the range specified above, the situation already involves marked restraints in respect of germs to be detected.

SUMMARY OF THE INVENTION

[0010] An object of the present invention is to afford a gamma-sterilisable nutrient medium for the detection of microorganisms in hydrogen peroxide-bearing air or on hydrogen peroxide-bearing surfaces, which does not suffer

from the above-indicated disadvantages and which affords enhanced operating results.

[0011] Another object of the present invention is to provide a gamma-sterilisable nutrient medium for the detection of microorganisms, which affords enhanced stability thereby facilitating storage and despatch and also providing a long potential period of use.

[0012] In accordance with the principles of the present invention the foregoing and other objects are now attained by a gamma-sterilisable nutrient medium based on casein soya peptone agar for the detection of microorganisms in hydrogen peroxide-bearing air or on a hydrogen peroxide-bearing surface, with the addition of between 2 and 10% by weight of sodium thioglycolate, between 5 and 20% by weight of sodium bisulfite and between 10 and 30% of sodium thiosulfate, in each case with respect to the agar. It was surprisingly found that agar medium based on casesin soya peptone agar neutralises hydrogen peroxide in levels of concentration as occur when collecting air-borne germs in isolators with hydrogen peroxide residual content, when the above-outlined additions are implemented.

[0013] Preferably the casein soya peptone agar employed is the Microbial Content Test Agar (MCT Agar; Difco 0553-07-4) which comprises casein soya peptone with the addition of sorbitan monooleate=Tween 80.RTM. and lethicin. Those media are also pH-stable by buffering in the pH-range of between 7.1 and 7.5.

[0014] Conventional buffering with phosphate buffer alone results in precipitation phenomena so that the medium has poor growth properties. As is known that can be avoided by buffering with MOPS (morpholinopropane sulfonic acid). MOPS however is very expensive. Surprisingly however it has now been found that partial replacement of phosphate by MOPS is possible without involving growth-reducing precipitation phenomena. Preferably, of the total amount of buffer between 20 and 50% is used in the form of MOPS, in which case MOPS is added firstly and thereafter the balance of between 50 and 80% of the total buffer content is slowly added, as phosphate buffer.

[0015] The pH-indicators which are usually added, bromocresol purple and bromothymol blue, are destroyed by gamma irradiation at between 16 and 25 kgray, with the consequence of the agar being of a gray appearance, which causes substantial difficulties in terms of evaluating white to gray colonies. It was surprisingly found that this can be prevented by the addition of polyvinylpyrrolidone in an amount of between 10 and 50%, preferably between 30 and 45%, with respect to the agar. The blue-violet to blue-green color is then maintained, which substantially facilitates evaluation of and checking for grown colonies.

[0016] Surprisingly, the hydrogen peroxide-neutralising action of the nutrient media according to the invention can be boosted by the addition of between 0.05 and 0.25% of pyruvate. These levels of concentration which are substantially lower in comparison with the Baird-Parker medium are of economic importance, because of the high price of sodium pyruvate.

[0017] Media according to the invention, buffered in the range of between pH 6.8 and 7.4, with the above-specified pH-indicators and the addition of sodium pyruvate and polyvinylpyrrolidone, are stable for 6 months, which substantially facilitates storage and shipping, and also guarantees that the customer has a long potential period of use. Those media are further capable of neutralising 2% H.sub.2O.sub.2-solutions which are applied directly and permitting subsequent growth of microorganisms. In comparison for example normal soya casein peptone agar no longer permits germ growth after exposure with only 0.02% hydrogen peroxide.

[0018] In addition, for better growth of germs which were damaged by drying out, for example in air or on a surface, it is possible to add betaine, glycine, cystine, proline and asparagine.

[0019] The Examples set out hereinafter further specifically illustrate the invention.

PREFERRED EMBODIMENTS

Example 1

[0020] Medium With Color Indicator for Applying to H.sub.2O.sub.2-Bearing Surfaces

1 Basic medium Microbial Content Test Agar 23 g (Difco 0553-07-4 = MCT Agar) Agar-agar (comprising casein soya peptone, 12 g common salt, lecithin, sorbitan-monooleate and agar) Polyvinylpyrrolidone (PVP 360) betaine 10 g (Sigma B3501)0.03 Betaine (Sigma B3501) 0.03 g L-glycine (Merck 104201) 0.05 g L-cystine (Merck 1028136) 0.025 g L-proline (Merck 107434) 0.025 g Pyruvic acid, Na-salt (Merck 106619) = 0.25 g sodium pyruvate L-asparagine (Merck 101565) 0.025 g Glucose (Merck 107074) 2.5 g Sodium thioglycolate (Sigma T0632) 1.0 g Sodium disulfite (Merck 106528) 2.5 g Sodium thiosulfate (Merck 106516) 6.0 g Bromocresol purple (Merck 103025) 0.025 g Bromothymol blue (Merck 103026) 0.025 g Aqua dest ad 1 liter Adjust pH to 7.3 .+-. 0.2, autoclave for 15 min at 121.degree. C. and after cooling add in sterile filtered condition: Yeast extract 2.5 ml (Marcor, 10 g yeast extract stirred cold into 100 ml VE-water and sterile-filtered) Phosphate buffer pH 7.3 1 molar solution 20 ml MOPS buffer pH 7.3 4 molar solution 6 ml (Sigma M1254, morpholinopropane sulfonic acid) (first add MOPS, thereafter phosphate slowly) L-ascorbic acid (Na-salt Sigma A7631, 1 g in 2 0.5 ml ml VE-water

Example 2

[0021] Medium Without Color Indicator for the Detection of Air-Borne Germs in H.sub.2O.sub.2-Bearing Isolator Air

2 Basic medium Microbial Content Test Agar 23 g (Difco 0553-07-4) Agar-agar 12 g Betaine (Sigma B3501) 0.03 g L-glycine (Merck 104201) 0.05 g L-cystine (Merck 1028136) 0.025 g L-proline (Merck 107434) 0.025 g Pyruvic acid, Na-salt (Merck 106619) = sodium 0.25 g pyruvate L-asparagine (Merck 101565) 0.025 g Glucose (Merck 107074) 2.5 g Sodium thioglycolate (Sigma T0632) 1.0 g Sodium disulfite (Merck 106528) 2.5 g Sodium thiosulfate (Merck 106516) 6.0 g Aqua dest ad 1 liter Adjust pH to 7.3 .+-. 0.2, autoclave for 15 min at 121.degree. C. and after cooling add in sterile filtered condition: Yeast extract 2.5 ml (Marcor, 10 g yeast extract stirred cold into 100 ml VE-water and sterile-filtered) Phosphate buffer pH 7.3 1 molar solution 20 ml MOPS buffer pH 7.3 4 molar solution 6 ml (Sigma M1254, morpholinopropane sulfonic acid) (first add MOPS, thereafter phosphate slowly) L-ascorbic acid (Na-salt Sigma A7631, 1 g in 2 ml 0.5 ml VE-water Cast in agar strips for air-borne germ collecting apparatus and subject to .gamma.-sterilisation (dose 16 - 25 kgray).

Example 3

[0022] Microbial Content Test Agar

[0023] Difco without additives

Example 4

[0024] Soy Bean Casein Digest Agar

[0025] Difco with 1% (10 g/L) additional sodium pyruvate

Example 5

[0026] D/E-Agar

[0027] Difco without additives.

[0028] All five kinds of agar, for testing their capacity for the neutralisation of H.sub.2O.sub.2, are subjected to the action of 100 microliters of H.sub.2O.sub.2-bearing solutions with 10 ppm of 0.02% H.sub.2O.sub.2, 0.5% H.sub.2O.sub.2, 1% H.sub.2O.sub.2 and 2% (20,000 ppm) H.sub.2O.sub.2. Thereafter the level of H.sub.2O.sub.2 concentration on the agar surface is measured with peroxide test strips (Merck) (Table 1). While the agar according to the invention still neutralises 2% (20,000 ppm) H.sub.2O.sub.2, the basic medium MCT alone is not capable of completely neutralising 10 ppm. Kinds of agar which are known from the literature (Examples 4 and 5) can already no longer completely neutralise 0.5% H.sub.2O.sub.2.

[0029] After exposure with H.sub.2O.sub.2 with inoculation of Staphylococcus aureus ATCC 6538 with 10-100 colony-forming units the possible growth after H.sub.2O.sub.2 exposure is investigated (Table 2).

[0030] The agar additives according to the invention as set forth in Examples 1 and 2 permit germ growth even after exposure with high H.sub.2O.sub.2 amounts while the basic agar used already exhibits marked restraints upon growth, due to 10 ppm H.sub.2O.sub.2. The variants which are known in the literature with pyruvate additive alone or D/E-agar in known form neutralise rather more H.sub.2O.sub.2 but they also already exhibit very marked growth restraints from 0.5% H.sub.2O.sub.2.

3TABLE 1 H.sub.2O.sub.2 concentration in the agar after application of 100 microliters of H.sub.2O.sub.2 solutions Soybean casein digest Concentra- MCT agar with 1% tion of the Agar Agar Example 3 pyruvate D/E agar applied Example 1 Example 2 (standard Example 4 Example 5 H.sub.2O.sub.2 solution (invention (invention) comparison) (literature) (comparison) 10 ppm 0 ppm 0 ppm 1-2 ppm 0 ppm 0 ppm 0.02% 0 ppm 0 ppm 30 ppm 0 ppm 0 ppm 0.5% 0 ppm 0 ppm >100 ppm 2-5 ppm 2-5 ppm 1.0% 0 ppm 0 ppm >100 ppm 5-10 ppm 10 ppm 2.0% 0 ppm 0 ppm 20-30 ppm 30 ppm (=20000 ppm)

[0031]

4TABLE 2 Growth of Staph. aureus 6538 after H.sub.2O.sub.2 exposure - inoculum of 10-100 colony-forming units (CFU) per agar surface (Petri dish agar strip contact slide) Soybean casein digest Concentra- MCT agar with 1% tion of the Agar Agar Example 3 pyruvate D/E agar applied Example 1 Example 2 (standard Example 4 Example 5 H.sub.2O.sub.2 solution (invention (invention) comparison) (literature) (comparison) 0 = control 68 CFU 73 CFU 61 CFU 71 CFU 62 CFU 10 ppm 63 CFU 74 CFU 18 CFU 68 CFU 73 CFU 0.02% 71 CFU 64 CFU 0 CFU 62 CFU 65 CFU 0.5% 61 CFU 59 CFU 0 CFU 12 CFU 14 CFU 1.0% 65 CFU 67 CFU 0 CFU 0 CFU 0 CFU 2.0% 69 CFU 62 CFU 0 CFU 0 CFU 0 CFU (=20000 ppm)

[0032] All 5 kinds of agar are cast in agar strips for RCS Highflow air-borne germ collecting apparatuses.

[0033] Thereafter in each case air samples were collected in parallel in an isolator with a comparatively high H.sub.2O.sub.2 residual loading and in an isolator ventilated overnight with a low H.sub.2O.sub.2 residual loading. For comparison purposes in each case non-loaded air was collected from a clean bench in a clean room. Inoculation with an inoculum of 10-100 colony-forming units of Staph. aureus ATCC 6538 was effected in each case directly after the air-borne germ collection procedure in order to anticipate a possible reduction in the H.sub.2O.sub.2 collected in the agar upon being left to stand. In that way the H.sub.2O.sub.2 loading should correspond to that which is found with a possible air-borne germ directly at the collection procedure. It will be seen from Table 3 that a normal standard agar permits germ growth after exposure with normal air without H.sub.2O.sub.2, but no longer in H.sub.2O.sub.2-loaded air, irrespective of the concentration. The agar types 4 and 5 known from the literature already exhibit marked weaknesses in performance in the isolator 1 at a relatively high level of H.sub.2O.sub.2 residual concentration while the agar types in accordance with the invention as set forth in Examples 1 and 2, as was to be expected from the results from Table 1, neutralise even relatively high levels of H.sub.2O.sub.2 concentration and permit unrestrained growth. The results are summarised in Table 3.

5TABLE 3 Air-borne germ measurements in the isolator. 1000 L H.sub.2O.sub.2-bearing isolator air is collected with an RCS air-borne germ collector and the strips thereafter inoculated with S. aureus 6538. Comparison non-loaded clean bench air. 1000 L air 1000 L air 1000 L clean isolator 1 isolator 2 bench air high H.sub.2O.sub.2 low H.sub.2O.sub.2 without H.sub.2O.sub.2 residual residual residual Agar type concentration concentration concentration Agar Example 1 86 92 83 (invention) Agar Example 2 81 79 88 (invention) MCT agar 0 0 91 Example 3 (standard comparison) Soybean 11 74 83 casein digest with 1% pyruvate Example 4 (literature) D/E-agar 7 92 94 Example 5 (comparison)

[0034] Implementation of an application of H.sub.2O.sub.2-loaded surfaces in the isolator in dependence on the storage time of the agar used clearly shows in Table 4 the substantially better level of stability of the nutritive properties of the medium according to the invention as set forth in Example 1 in comparison with the D/E-standard agar known from the literature. With standard MCT agar the nutritive properties are admittedly more stable, but at a somewhat lower level in relation to Staph. aureus. In the case of the anaerobic germ C. sporogenes it will be seen that the lack of

H.sub.2O.sub.2-neutralisation of MCT agar prevents the growth of anaerobes after H.sub.2O.sub.2 exposure while that is still readily possible with the agar according to the invention. In the case of anaerobic germs incubation is always anaerobic after inoculation.

[0035] The incubation temperature for all bacteria is adjusted in accordance with USP at 32.5.degree. C..+-.2.5.degree. C.

6TABLE 4 Growth of S. aureus 6538 and C. sporogenes after application of H.sub.2O.sub.2-fumigated isolator surfaces Agar Agar Agar type Growth of type Growth of type Growth of age S. C. sporo- age S. C. sporo- age S. C. sporo- 4 wks aureus genes 3 mon aureus genes 6 mon aureus genes Agar Ex 71 68 Agar Ex 88 92 Agar Ex 69 98 1 (Inv) 1 (Inv) 1 (Inv) MCT agar 58 0 MCT agar 49 0 MCT agar 52 0 Ex 3 Ex 3 Ex 3 (standard (standard (standard compari- compari- compari- son) son) son) D/E-agar 68 16 D/E-agar 23 0 D/E- agar 0 0 Ex 5 Ex 5 Ex 5 (compari- (compari- (compari- son) son) son)

[0036] Table 5 summarises the result of growth of an entire germ spectrum after passing through 1000 liters of H.sub.2O.sub.2-bearing isolator air in comparison in each case with 1000 liters of air from a clean bench without isolator. The growth of gram-positive cocci (S. aureus), gram-positive sporogenic rods (B. subtilis), anaerobic sporogenes (C. sporogenes), gram-negative enterobacteriaceae (E. coli), gram-negative non-fermenters (P. aeruginosa), yeasts (C. albicans) and fungi (A. niger) is investigated. On MCT agar and agar according to the invention all germs grow equally well after exposure with normal air (clean bench air). After exposure of H.sub.2O.sub.2-bearing isolated air all germs grow in a comparable number on the agar according to the invention as set forth in Example 2, while on MCT agar after exposure with H.sub.2O.sub.2-bearing isolator air no growth whatsoever can be found with all germs.

7TABLE 5 Growth after collecting H.sub.2O.sub.2-bearing isolator air and clean bench air without H.sub.2O.sub.2 respectively 1000 L 1000 L clean isolator air bench air Agar MCT agar Agar MCT agar Germ Example 2 Example 3 Example 1 Example 3 S. aureus 85 0 82 76 E. coli 43 0 38 41 P. aerunginosa 61 0 56 58 B. subtilis 28 0 23 26 C. sporogenes 24 0 19 21 C. albicans 16 0 1 18 A. niger 38 0 42 36

[0037] The culture media according to the invention can be gamma-sterilised without problems.

[0038] It will be appreciated that the above-described Examples have been set forth by way of illustration of the present invention and that various modifications may be made therein without thereby departing from the spirit and scope of the invention.

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The Contents of Case *"10623241US20060320"*

Qnum	Query	DB Name	Thesaurus	Operator	Plural
Q1	microbial near5 culture media	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q2	bromocresol purple	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q3	(casein soy peptone agar)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q4	(casein near5 soy)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q5	(peptone near5 agar)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q6	sodium pyruvate	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q7	bromothymol blue	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q8	Q4 near5 Q7	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q9	Q4 and Q7	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q10	Q5 and Q9	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q11	Q6 and Q10	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q12	Q2 and Q11	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q13	Q1 and Q12	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q14	hydrogen peroxide near5 resistant	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q15	bacteria or microorganisms or yeasts or fungi	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q16	Q14 and Q15	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q17	Q12 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q18	Q1 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q19	Q2 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q20	Q4 and Q19	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q21	Q5 and Q19	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q22	Q4 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q23	Q5 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q24	Q6 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Q25	Q7 and Q16	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q26	Q19 and Q25	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q27	Q1 and Q14	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q28	Q15 and Q2	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
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Q40	Q5 and Q38	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q41	Q6 and Q38	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q42	Q7 and Q38	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q43	sterilize near5 (gamma rays or gamma radiation)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q44	Q43 and Q1	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q45	Q16 and Q43	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q46	6908745.pn.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q47	435/253.6.ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q48	Q43 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q49	Q1 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q50	Q2 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q51	Q4 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q52	Q5 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q53	Q6 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q54	Q7 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Q55	Q14 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q56	Q15 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q57	Q16 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q58	Q28 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q59	Q37 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q60	Q43 and Q47	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q61	6136554.pn.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q62	5968807.pn.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q63	Q2 and Q62	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q64	Q7 and Q63	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q65	Q14 and Q64	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q66	Q6 and Q64	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q67	Q43 and Q64	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q68	(Q2 or Q7) and Q61	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q69	(Q2 and Q7) and Q61	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q70	(Q2) and Q61	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q71	(Q7) and Q61	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q72	Q62 and (pyruvate or pyruvic acid)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q73	Q61 and (pyruvate or pyruvic acid)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q74	Q62 and (hydrogen peroxide)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q75	Q36 and Q14	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q76	Q75 and Q2	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q77	Q3 and Q76	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q78	Q4 and Q76	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q79	Q5 and Q76	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q80	Q6 and Q76	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q81	Q7 and Q76	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q82	Q75 and Q3	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q83	Q75 and Q4	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q84	Q75 and Q5	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q85	Q75 and Q6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Q86	Q75 and Q7	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q87	Q61 and hydrogen peroxide	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q88	Q1 and Q2	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q89	Q88 and Q62	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q90	GAs near5 steriliz$6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q91	Q85 and Q90	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q92	Q88 and Q90	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q93	Q89 and Q90	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q94	bacteria and Q91	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q95	(hydrogen peroxide vapor resistant)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q96	Q36 and Q95	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q97	Q35 and Q95	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q98	Q94 and Q97	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q99	Q95 same bacteria	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q100	Q95 same yeast	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

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